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TP248.13.C87
Vol. No 9/26
4L5804

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Please provide the following reference(s):

1) Curr Opin Biotechnol. 1990 Oct;1(1):36-47.

**Mammalian cell expression.**

**Gorman CM.**

2) Biotechnology (N Y). 1990 Jan;8(1):54-8.

**Spin filter perfusion system for high density cell culture: production of recombinant urinary type plasminogen activator in CHO cells.**

**Avgerinos GC, Drapeau D, Socolow JS, Mao JI, Hsiao K, Broeze RJ.**

3) Biotechnology (N Y). 1995 Apr;13(4):389-92.

**Production of recombinant proteins in Chinese hamster ovary cells using a protein-free cell culture medium.**

**Zang M, Trautmann H, Gandor C, Messi F, Asselbergs F, Leist C, Fiechter A, Reiser J.**

4) Mol Cell Biol. 1985 Jul;5(7):1750-9.

**Coamplification and coexpression of human tissue-type plasminogen activator and murine dihydrofolate reductase sequences in Chinese hamster ovary cells.**

**Kaufman RJ, Wasley LC, Spiliotes AJ, Gossels SD, Latt SA, Larsen GR, Kay RM.**

1180357

Thank you,

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# Mammalian cell expression


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Current Opinion in Biotechnology 1990, 1:36–47


## Introduction

The expression of recombinant genes in mammalian cells has become a cornerstone technique of biotechnology. It requires a knowledge of such diverse techniques as gene expression, DNA transfer, amplification of DNA, and the processing, secretion and production of proteins. The successful production of antibodies (Fig. 1) in mammalian cells, the focus of this brief review, began with the application of basic knowledge of transcription of immunoglobulin genes to the expression of recombinant antibodies in cells. New gene-transfer techniques and protein engineering then facilitated the production of a variety of recombinant antibodies. More recently, these antibodies have been used to elucidate the steps involved in antibody assembly and secretion, and to enable production of completely new proteins, such as hybrids between portions of the heavy-chain constant region of Ig and unrelated proteins.

As this review focuses on the expression of recombinant antibodies, other important advances in the past year or so in the broader field of mammalian gene expression are covered by annotated references and patents.

## Control of immunoglobulin gene expression

One of the first cellular expression systems identified as cell-type specific was the immunoglobulin gene system. The expression of these genes is restricted to B lymphocytes. *Cis*-acting enhancer sequences, located within the J–C intron of the immunoglobulin heavy-chain genes form a major component of cell-type-specific control mechanisms (Fig. 2). The *trans*-acting protein, NF-κB, interacts with this enhancer sequence and facilitates expression of the immunoglobulin genes in mature plasmacytomas. The mechanism involved in cell-type specificity is not clear because, unlike transfected immunoglobulin heavy-chain genes, their endogenous counterparts function in myeloma cells without the immunoglobulin heavy-chain gene enhancer. Interestingly, transfected immunoglobulin genes also require the heavy-chain enhancer for continued expression in pre-B-cells [1••]. Even though the NF-κB binding site may not be required for expression in pre-B-cells, other *cis*-acting sequences might be needed. Nelms *et al.* [2••] have identified a region upstream of the NF-κB binding site, within the enhancer, that is stimulated in pre-B-cells but not mature plasmacytomas. The presence of newly identified 3′ enhancers [3•,4•] could explain the apparent NF-κB independence of the endogenous gene but not transfected genes. These B-cell-specific enhancers are located 25 kb

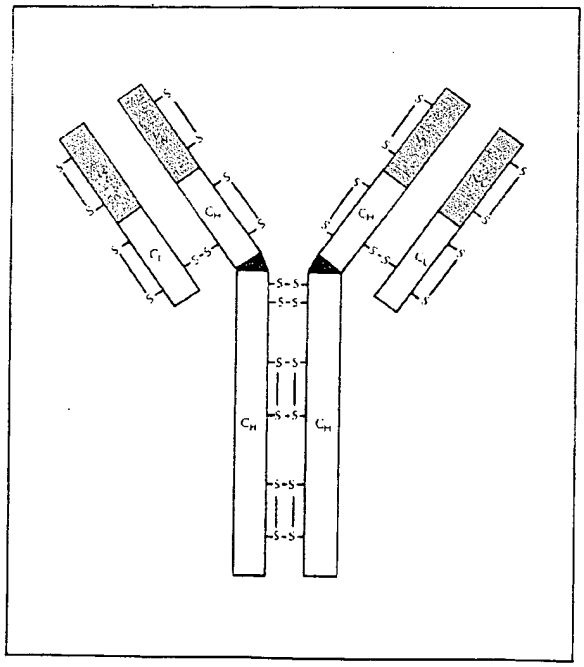

**Fig. 1.** Schematic diagram of an IgG antibody. $V_H$, heavy-chain variable region; $C_H$, heavy-chain constant region; $V_L$, light-chain variable region; $C_L$, light-chain constant region. The hinge region is shown as black. Inter- and intramolecular disulfide bridges are shown.

**Abbreviations**

**ADCC**—antibody-dependant cellular cytotoxicity; **CDR**—complementary-determining regions; **DHFR**—dihydrofolate reductase; **EBV**—Epstein-Barr virus; **hyg**—hygromycin; **gpt**—xanthine phosphoribosyltransferase; **LPV**—lymphotropic virus; **MTX**—methotrexate; **neo**—neomycin; **PCR**—polymerase chain reaction; **SV** simian virus.

3'of the κ light-chain gene locus and 10 kb 3' of the heavy-chain gene locus. They contain multiple enhancer core regions including motifs homologous to the enhancers of simian virus (SV)40, lymphotropic virus (LPV), NF-κB and interferon. In experiments, the 3' enhancers show apparent independence of the heavy-gene chain enhancers. It would seem that the heavy-chain gene enhancer is more complex than once thought. This region also has promoter activity, when assayed in the absence of the heavy-chain gene promoter. The octamer binding site (see below) is important for this activity [5•].

The immunoglobulin promoter (Fig. 2) also contributes to the specificity of expression. The identification of a specific octamer sequence, ATGCAAAT, within this transcriptional control region has enabled detailed analysis of factors involved in control of these genes. Heterologous expression in B cells is greatly increased by the presence of this motif. Three *trans*-acting proteins bind to this sequence, Oct-1 also known as OTF-1, Oct 2A and Oct 2B. Oct-1 is ubiquitous and is therefore thought not to be responsible for tissue-specific expression, while Oct2A, the predominant B-cell-specific factor, and Oct2B, a minor factor, are responsible at least in part for the cell-type-specific expression of immunoglobulin genes [6•]. The mode of specificity provided by Oct-2 proteins is unclear. The level of Oct-2 in lymphoid cells does not correspond to the level of immunoglobulin gene transcription *in vitro* in lymphoid extracts. Reconstitution experiments show that while the Oct-2 binding proteins are required to enable transcription in non-lymphoid extracts, these proteins are not sufficient; however, Oct-1 by itself can restore this activity [7•].

These octamer transcription factors also bind to a heptamer element, CTCATGA, which is located 2–22 bp upstream of the octamer sequence within the heavy-chain promoter. It is possible that differential binding to the heptamer and octamer sequences occurs during differentiation of B cells. Kemler *et al.* [8•] suggest that there is a cooperative interaction between heptamer and octamer binding. As less Oct-2 is present in pre-B-cells, such cooperative interaction, facilitated by multiple binding sites, could be advantageous in the process prior to differentiation. The presence of multiple transcription control sequences suggests that a complex pattern of transcriptional events is required before B-cell activation can take place.

Once these enhancer and promoter factors, or their genes, have been isolated they could become useful in the recombinant expression of antibodies as transfected genes are also under their control [6•,8•,P1•].

## Production of mouse–human chimeric antibodies

As gene transfer into myeloma cells has progressed, it has become feasible to consider the development of recombinant antibodies. Because most monoclonal antibodies used to date as therapeutics are of mouse origin, they may be ineffective at recruiting human immune effector functions, such as complement-dependant cytotoxicity and antibody-dependant cellular cytotoxicity (ADCC), and may even elicit an immune response. For these reasons, the production of mouse–human hybrid or chimeric antibodies has flourished. The basic approach is to replace the constant regions of a mouse antibody with a human constant region while maintain-

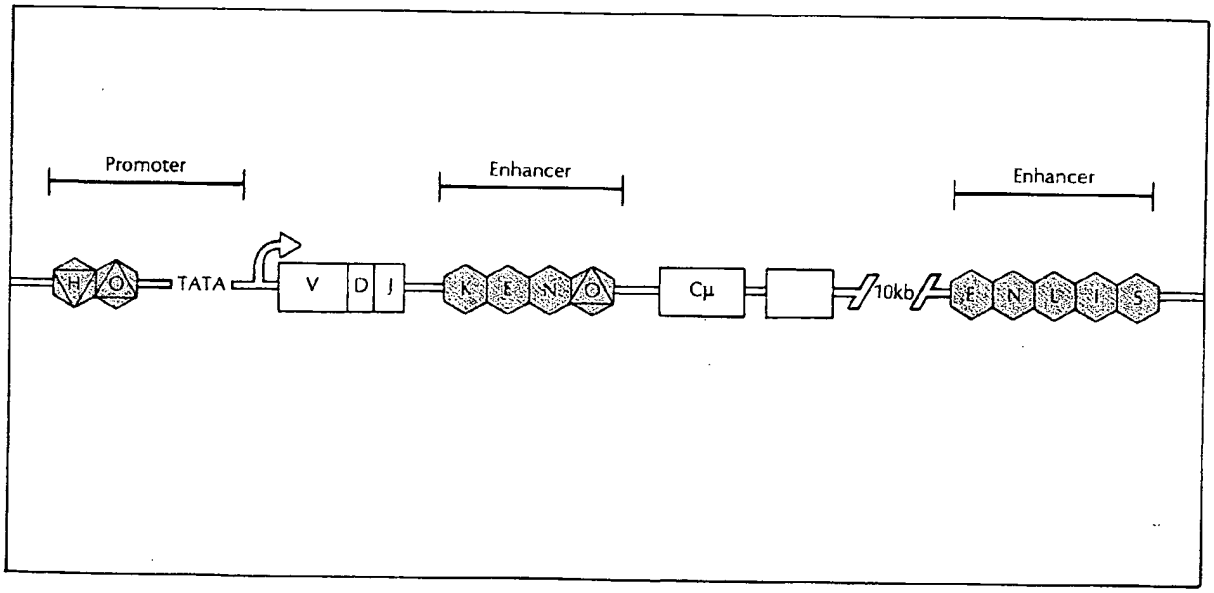


**Fig. 2.** Transcriptional control elements of the heavy-chain μ gene. The promoter contains both a heptamer (H) and an octamer (O) motif for binding *trans*-acting factors. The heavy-chain gene enhancer, located within the intron, contains an octamer motif as well as NF-κB (N) and KBF-A (K) binding sites and an E box (E). The enhancer located 10 kb 3' of the constant region contains an E box, an NF-κB binding site and sequences homologous to the enhancers of lymphotropic virus (L), interferon (I), and simian virus 40 (S).

ing the antigen-binding sites of the mouse variable regions (Fig. 3). This experimental design requires isolation of the variable region of the desired mouse antibody gene. To facilitate this endeavor Orlandi *et al.* [9••] have recently designed a set of synthetic oligonucleotide primers for polymerase chain reaction (PCR) amplification of the cDNAs of mouse immunoglobulin heavy- and light-chain variable domains. Through a systematic survey of the aligned sequences of the $V_H$ and $V_\kappa$ genes, conserved nucleotides flanking the 5′ end of these genes have been identified. Specific restriction enzyme sites included within these primers simplify the process of cloning these variable sequences.

Typical transfection expression mouse–human fusion vectors are shown in Fig. 4 [9••,10•]. Immunoglobulin transcriptional control regions, such as the enhancers and promoter sequences described above, are used in these vectors as is the immunoglobulin protein signal sequence. Unique restriction sites for subcloning the desired mouse variable regions follow this signal. Genomic DNA is most often used for the heavy or light constant domains and, therefore, it is not necessary to supply the enhancer in the J-C intron. Electroporation is the method of choice for gene transfer into myeloma cells. As cotransfection efficiencies with this technique are poor, a separate selectable marker is often included on each heavy-chain vector and each light-chain vector. The preferred selective markers for myeloma cells are neomycin (neo), xanthine phosphoribosyltransferase (gpt) and hygromycin [9••,10•,11•,12•].

An alternative method of producing chimeric antibodies with myeloma cells is described by the Bristol-Myers Company [P2••]. It uses gene targeting via homologous recombination in antibody-producing cells. For example, once a hybridoma line that is producing a desired antibody has been identified, *in vivo* recombination is used to replace the mouse constant region with a human constant region. In this example, the target sequence that is used to initiate the recombination results in replacement of the mouse constant region with the human sequences (Fig. 5) contains the murine sequence encoding the fourth joining region as well as diversity and switch regions, the immunoglobulin heavy-chain enhancer and intronic sequences 5′ to the switch region. The vector

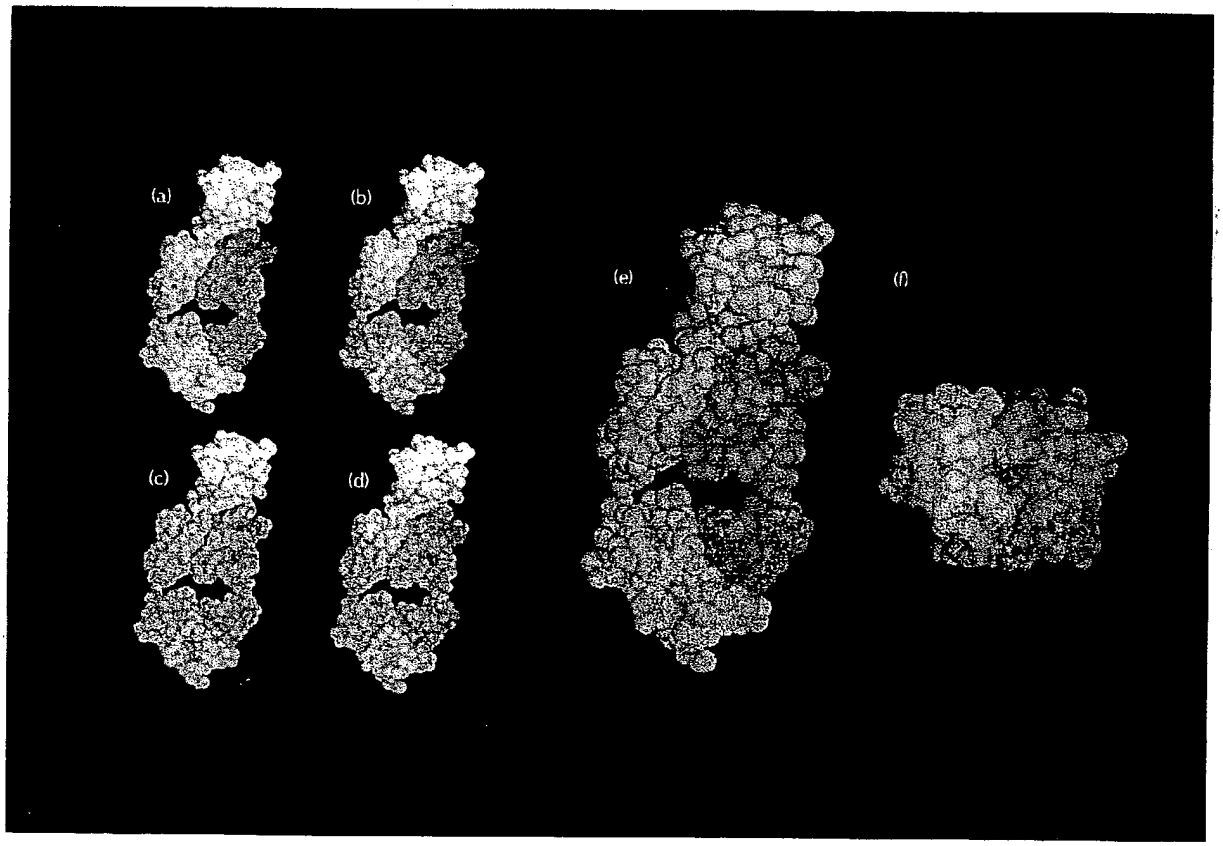


**Fig. 3.** Computer representation of chimeric and humanized antibodies. Variants of an anti-lysozyme antibody (a–d) and humanized Fab (e,f) close up (α carbon trace only). (a) Mouse Fab: $V_H$-$C_H1$, green; $V_L$-$C_L$, yellow; lysozyme, pink. (b) Chimeric Fab: mouse $V_H$, green; human $C_H1$, blue; mouse $V_L$, yellow; human $C_L$, purple; lysozyme, pink. (c) Human Fab: $V_H$-$C_H1$, blue; $V_L$-$C_L$, purple; lysozyme, pink. (d) Humanized Fab: $V_H$-$C_H1$, blue; mouse $V_H$ CDRs, green; $V_L$-$C_L$, purple; mouse $V_L$ CDRs, green; (e) A view with lysozyme present. Human $V_H$-$C_H1$, blue; human $V_L$-$C_L$, purple; mouse light-chain CDRs, yellow; mouse heavy-chain CDRs, green; lysozyme, pink. (f) Right side is a view without lysozyme and has been rotated by 90 °about the X-axis to show the CDRs. CDR, complementary-determining regions; $C_H$, heavy-chain constant region; $C_L$, light-chain constant region; $V_H$, heavy-chain variable region; $V_L$, light-chain variable region.

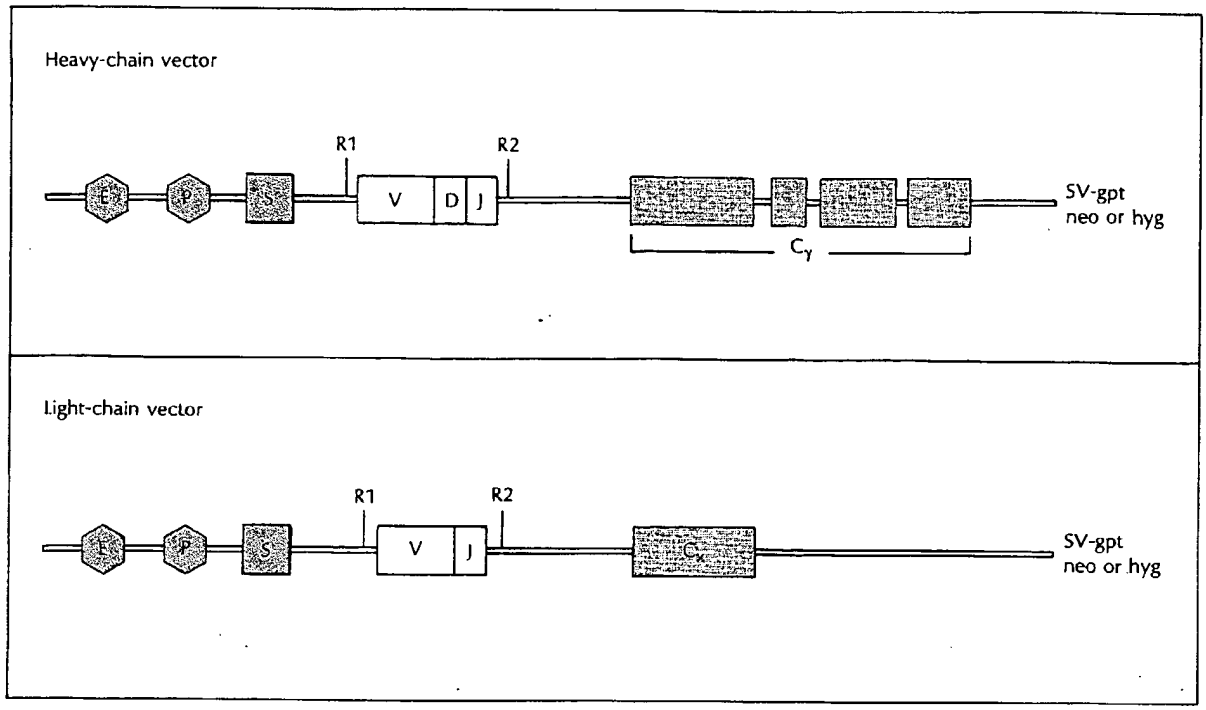


**Fig. 4.** Chimeric expression vectors. Typical vectors use the immunoglobulin enhancer (E), promoter (P), and signal (S) sequence from the immunoglobulin genes. These vectors include unique restriction sites (R1 and R2) to facilitate the subcloning of mouse variable (V) region cDNAs. The mouse variable region is followed by human genomic DNA encoding the constant (C) region. Diversity (D) and joining (J) regions are also shown along with the simian virus (SV) selective markers gpt, neo and hyg.

also includes the human IgG constant region. Vector DNA is linearized within the target region to facilitate recombination. Transfected cells are screened for human IgG protein.

Though most efforts to produce recombinant antibodies use mycloma cells, it is also possible to produce them in non-lymphoid cells. Two examples were cited in recently published patents. Eli Lilly [P3•] describe chimeric cDNAs created by fusing the mouse variable region to the human constant region; these cDNAs are introduced into expression vectors containing the BK virus enhancer followed by the adenovirus major late promoter. An intron is included in these vectors 3′ of the cDNAs. Traditional selection markers are used in conjunction with the amplifiable marker dihydrofolate reductase (DHFR). As the BK enhancer is positively regulated by adenovirus E1A proteins, cells constitutively expressing E1A, such as AV12 (ATCC CRL 9595), show the best production of IgG protein. Co-transfection of these cells with vectors encoding both the chimeric heavy-chain and chimeric light-chain cDNAs results in secretion of chimeric antibodies.

In addition to expression of IgG proteins in non-lymphoid cells, Celltech [P4••] describe experiments in which the more complex immunoglobulin protein, IgM, is produced in non-lymphoid cells. Assembly of these molecules in lymphoid cells is thought to require the presence of J-chains (Fig. 6). However, recombinant polymeric IgM antibodies form in non-lymphoid cell even in the absence of J-chains. Expression vectors containing

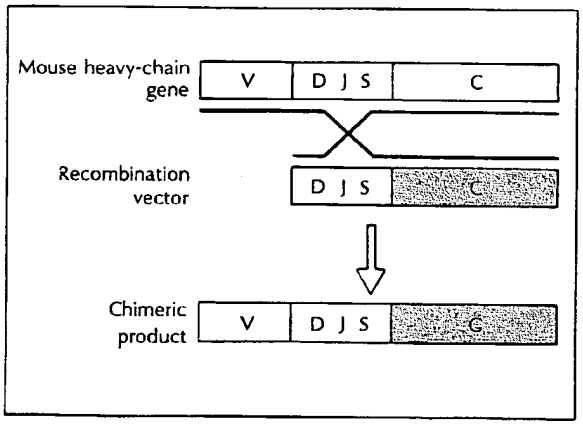


**Fig. 5.** Producing chimeric antibodies using recombination. Starting with an intact mouse heavy-chain gene sequence the mouse constant region can be replaced with the human constant region. Variable (V), diversity (D), joining (J), switch (S) and constant (C) region are indicated. In this example, the sequences conserved for homologous recombination are D, J and S. The recombination product contains mouse sequences for V, D, J and S regions with human sequences for the C region.

the *Drosophilia* heat-shock promoter, hsp70, were tested in four types of non-lymphoid cells. Secretion of polymeric IgM molecules ranged from 5 to 600 ng/ml, compared with 3500 ng/ml from a plasmacytoma cell line, and was greatest in a glioma cell line. The authors speculate that this is because of the secretory nature of glioma cells.

## Humanized and human antibodies

Chimeric antibodies as described above contain the entire mouse variable regions and as such may be recognized by the human immune system as foreign proteins. Techniques have therefore been developed to 'humanize' these chimeric antibodies further. The challenge is to change the amino-acid sequence from that characteristic of the mouse to a more human form while maintaining the antigen-binding capacity of the mouse complementary-determining regions (CDR) (Fig. 3). The pioneering work on humanizing antibodies has been carried out by Winter and colleagues at the MRC Laboratory of Molecular Biology in Cambridge.

A recent example is the production of a humanized antibody to the interleukin 2 receptor, anti-Tac, and the characterization of its antigen binding [13•,14•]. To attain a humanized antibody, sequence homology and molecular modeling are used to select a combination of mouse and human sequence elements that reduced the potential human immune response yet retain high-affinity binding. This approach assumes that the more homologous the human antibody is to the original mouse antibody, the fewer distortions will occur when combining mouse CDRs with the human framework. Therefore, as the Eu antibody (human heavy-chain subgroup I) is 57% identical to the mouse anti-Tac heavy chain variable region, its antibody framework was used and its CDRs were changed to those of the anti-Tac antibody. Computer graphic manipulation identified other framework residues that may influence the conformation of the CDRs. These residues were transferred to the antibody because they should help preserve the structure of the CDRs while still maintaining the human character of the protein. The humanized version of the anti-Tac antibody retains high levels of antigen binding and maintains the biospecificity of the murine antibody. This humanized antibody is functional in an ADCC assay while the mouse anti-Tac is not; this feature of the recombinant antibody could be important for its therapeutic success.

Another approach to minimizing the potential immune response that murine antibodies might elicit is to use human antibodies themselves; either mouse–human heterohybridomas or immortalized human B-cell lines provide the means of producing them but instability of antibody production is a severe limitation of these systems. Recently, however, two groups used such cells as a source of DNA for human antibodies. Nakatani *et al.* [15•] cloned genomic DNA for an IgM human antibody to *Pseudomonas* exotoxin A from an Epstein-Barr virus (EBV) transformed human B-cell. The human transcriptional control regions, including enhancers and promoter, directed expression of these genomic sequences in mouse myeloma cells, indicating a lack of species specificity for these sequences. Gillies *et al.* [16••] used a mouse-human heterohybridoma as the source of message coding for a human antibody to tetanus toxoid. A synthetic intron was introduced between the variable and constant regions of this cDNA. The fusion between these domains occurred at the level of RNA splicing within the transfected myeloma. Gillies *et al.* also describe the use of the DHFR gene as a selective marker for high-expressing cell lines when grown in methotrexate (MTX). Clones were isolated that contained up to a 100 copies of the vector-transferred genes, with levels ranging from 6 to 20 μg of antibody per ml of supernatant.

These studies describe an alternative way of achieving stable cell lines that produce intact human antibodies; it could have advantages over the mutagenesis approach required by protein engineering.

## Studies on antibody assembly

Recombinant antibody expression techniques have enabled studies to be performed that investigate the intracellular interactions between the various domains of the antibody molecules. These studies provide information that might be important in further improving the production of recombinant antibodies. Simon and Rajewsky [17••] have investigated whether the constant domains influence the antigen-binding sites by switching the $V_H$ domain onto the $C_L$ domain and the $V_L$ onto the $C_{H1}$ domain. Interchanging the two V domains did not interfere with the expression or secretion of antibodies and the switch of the V region to the C region of a different chain class did not affect antigen binding. No detectable evidence of the C domain acting on antigen binding through longitudinal inter-domain contacts was observed. One interpretation of this work is that V–V interactions mediate chain associations. Simon and Rajewsky suggest that *in vivo* chain pairing could be initiated by V pairing if the light chain first binds a heavy chain while the heavy chain is still complexed with the chaperone protein BiP. Though a complete discussion of how BiP may bind immunoglobulin proteins in order to facilitate their assembly is beyond the scope of this review, it is perhaps noteworthy that Nikaki *et al.* [18•] detect direct binding of non-secreted light chains to this chaperone protein. It was previously thought to bind only to the heavy chain.

The role of the hinge region in the formation of an antibody can now be investigated as this region is perturbed by these 'switched' mutants. Tan *et al.* [19•] address the role of the hinge region in the flexibility of the antibody complex. A correlation exists between the upper hinge length and the segmental flexibility of the chimeric antibodies studied. The position of the disulfide bridge between the heavy and light chains was not affected by either hinge shuffling or $C_H1$ exchange but the hinge region affected the assembly pattern of the heavy and light chains and therefore the complex that was secreted.

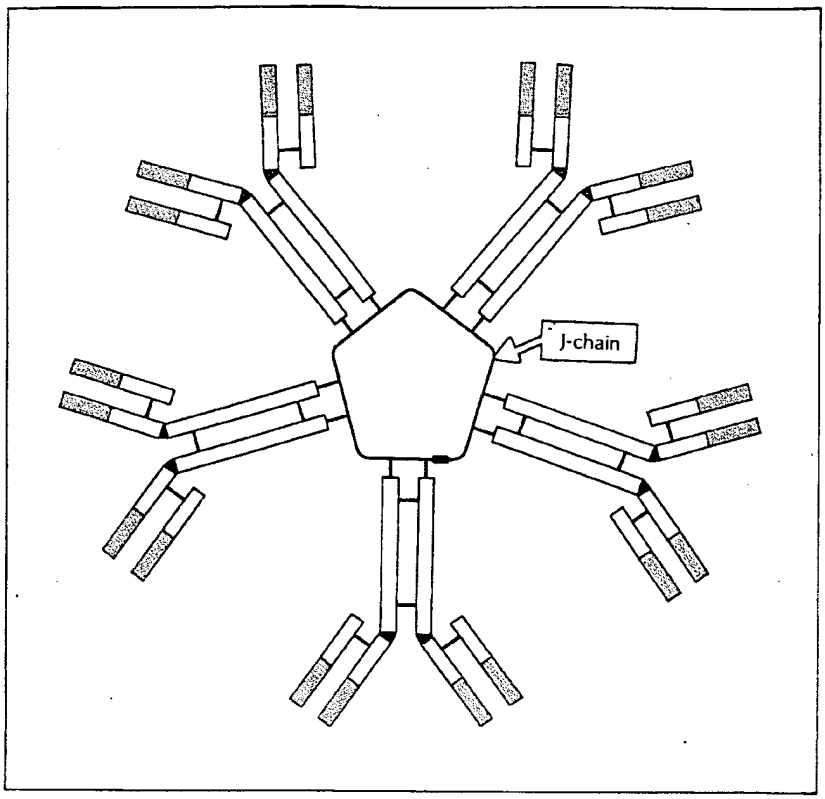


**Fig. 6.** Pentameric structure of IgM. The IgM chains are linked to form this complex structure through disulfide bonds. Though J chain has been indicated in the format of the structure, work described in this review indicates the structure can be formed without it.

Formation of the pentameric IgM complex (Fig. 6) has also been studied through recombinant expression. One fact to arise (already discussed above) is that J-chain genes do not appear to be required for pentameric assembly of IgM molecules. To study the assembly of these pentameric molecules Davis *et al.* [20•] have made several mutants replacing various cysteine residues with serine. These mutant genes were analyzed after transfection into a mouse hybridoma cell line. Conclusions from this work include: the Cys414–Cys414 bond between subunits stabilizes the pentameric molecule; this rigidity is necessary for activation of complement component C1.

## Chimeric proteins using immunoglobulin domains

One approach to chimeric antibodies not yet discussed is the ability to use variable regions from antibodies that have different specificities. By including such a combination of sequences it is possible to match bispecific antibodies [21•,P1•,P2••]. Alternatively, other functional domains can replace one or both of the variable regions to yield new proteins with wider functions. Such chimeric antibodies have been made using the toxin ricin or enzymes such as alkaline phosphatase [P1•,P2••].

Other examples of chimeric proteins that make use of immunoglobulin heavy-chain domains include CD4–Ig and T-cell-receptor–Ig molecules. CD4, the cellular receptor for HIV-1 has been produced as a soluble molecule. In this form, it has a short half-life; however, chimeric molecules made with either IgG [22•] or IgM [23•] heavy-chain constant regions have greater stability, with the half-life of CD4 increasing from 0.25 h to 113 h [22•]. Such studies provide useful information for the design of chimeric heavy-chain molecules, such as the production of the $V_a$ and $V_b$ subunits of the T-cell receptor [24•]. Deletion of the $C_H1$ domain increases secretion of molecules containing the heavy constant chain and expressed in the absence of light chain. Further, the pentameric formation of the chimeric IgM molecules results in molecules 1000-fold more active than IgG fusions, as assayed by binding of the complement receptor [23•].

## Acknowledgements

I wish to thank Len Presta, Protein Engineering Department, Genentech, Inc. for kindly providing the example of computer modeling for Fig. 3, Carol Morita for providing the graphic illustrations and Rob Arathoon for his work on the manuscript.

## Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest


1. •• PORTON B, ZALLER DM, LIEBERSON R, ECKHARDT LA: **Immunoglobulin heavy-chain enhancer is required to maintain transfected γ2A gene expression in a pre-B-cell line.** *Mol Cell Biol* 1990, 10:1076–1083.

To test whether the heavy-chain enhancer is required for expression in pre-B-cells a system was developed that allows initiation of enhancer-controlled expression followed by subsequent removal of the enhancer. The model uses the fact that pre-B-cells contain a recombinase while myelomas do not. Pre-B-cells are therefore capable of deleting the immunoglobulin heavy-chain gene enhancer. The conclusion is that heavy-chain genes transfected into pre-B-cells require the above enhancer to maintain their expression.

2. •• NELMS K, HIROMAS R, VAN NESS B: **Identification of second inducible DNA–protein interaction in the Kappa immunoglobulin enhancer.** *Nucl Acids Res* 1990, 18:1037–1043.

This paper discusses the identification of a transacting factor, in addition to NK-κB, that interacts with the κ enhancer. This new factor, κBF-A, contributes to the inducible function of the enhancer in activation of B cells.

3. •• PETTERSSON S, COOK GP, BURGGEMANN M, WILLIAMS GT, NEUBERGER MS: **A second B cell-specific enhancer 3′ of the immunoglobulin heavy chain locus.** *Nature* 1990, 344:165–168.

This work identifies a 3′ enhancer present in the heavy-chain locus that is analogous to sequences present in the mouse κ locus. It is suggested that this new enhancer could play a role in the activation of the translocated C-*myc* genes in rat immunocytomas, mouse plasmacytomas and Burkitt lymphomas.

4. •• MEYER KB, NEUBERGER MS: **The immunoglobulin κ locus contains a second, stronger B-cell-specific enhancer which is located downstream of the constant region.** *EMBO J* 1989, 8:1959–1964.

A novel enhancer is identified 9 kb downstream of the constant region of the κ locus. This enhancer is up to sevenfold stronger than the κ-intron enhancer and shows strong sequence homology to the lymphotropic papovavirus, heavy chain and κ intron enhancers.

5. • SU LK, KADESCH T: **The immunoglobulin heavy-chain enhancer functions as the promoter for Im sterile transcription.** *Mol Cell Biol* 1990, 10:2619–2624.

The immunoglobulin heavy-chain enhancer is shown to have promoter activity. The promoter activity from the enhancer sequence is dependent on the conserved octanucleotide ATTTGCAT.

6. • KEMLER I, SCHAFFNER W: **Octamer transcription factors and the cell type-specificity of immunoglobulin gene expression.** *FASEB J* 1990, 4:1444–1449.

The diversity of octamer transcription factors and their role in cell-type-specific expression of antibodies is discussed. Several transacting proteins seem to be able to recognize the motif ATGCAAAT. It is suggested that alternative splicing of a single transcript may yield a diverse family of proteins.

7. • JOHNSON DG, CARAYANNOPOULOS L, CORPRA JD, TUCKER PW, HANKE JH: **The ubiquitous octamer-binding protein(s) is sufficient for transcription of immunoglobulin genes.** *Mol Cell Biol* 1990, 10:982–990.

Two human pre-B-cell lines, containing extremely low levels of OTF-2, were found to express high levels of steady-state immunoglobulin heavy-chain mRNA *in vivo* and efficiently transcribe an immunoglobulin gene *in vitro*. These authors suggest that OTF-1 is sufficient for transcription of immunoglobulin genes.

8. • KEMLER I, SCHREIBAR E, MULLER MM, MATTHIAS P, SCHAFFNER W: **Octamer transcription factors bind to two different sequence motifs of the immunoglobulin heavy chain promoter.** *EMBO J* 1989, 8:2001–2008.

Two very different sequences present in the immunoglobulin heavy-chain promoter bind the same proteins, Oct 2A and Oct 2B, in addition to the ubiquitous protein Oct 1. Titration experiments show that there may be cooperative binding of the protein to these diverse elements.

9. •• ORLANDI R, GUSSOW DH, JONES PT, WINTER G: **Cloning immunoglobulin variable domains for expression by the polymerase chain reaction.** *Proc Natl Acad Sci USA* 1989, 86:3833–3837.

This paper discusses the use of PCR to clone a number of variable region cDNAs for insertion into chimeric antibody expression vectors.

10. • KAMEYANA K, IMAI K, ITOH T, TANIGUCHI M, MURIA K, KUROSAWA K: **Convenient plasmid vectors for construction of chimeric mouse/human antibodies.** *FEBS Lett* 1989, 244:301–306.

The heavy-chain vector contains the genomic sequence for human γ heavy-chain. Upstream of this region is a unique EcoRI site for insertion of any mouse variable region. This vector also includes a SV40-*gpt* transcription unit. The light-chain vector uses the neomycin gene as a selectable marker and contains a unique HindIII site for cloning the variable regions.

11. • HOOGEHBLOOM HR, RAUS JC, VOLCHAERT G: **Cloning and expression of a chimeric antibody directed against the human transferrin receptor.** *J Immunol* 1990, 144:3211–3217.

Chimeric cDNA containing the murine V region and the $C_H1$ region were cloned onto a genomic heavy chain and expressed following transfection with a similar light chain. The chimeric antibody retains the same antigen specificity as the murine antibody.

12. • LI YW, LOWRIE DK, THAMMANA P, MOORE GP, SHEARMAN CW: **Construction, expression and characterization of a murine/human chimeric antibody with specificity for hepatitis B surface antigen.** *Mol Immunol* 1990, 27:303–311.

Using vectors that had constant regions from genomic DNA, a murine human chimeric antibody for hepatitis-B virus was made. This chimeric antibody may be useful in the prevention of vertical transmission of hepatitis-B virus.

13. • QUEEN C, SCHNEIDER WP, SELICK HE, PAYNE PW, LANDOLF NF, DUNCAN JF, ARDVALOVIC NM, LEVIT M, JUNGHANS RP, WALDMAN TA: **A humanized antibody that binds to the interleukin 2 receptor.** *Proc Natl Acad Sci USA* 1989, 86:10029–10033.

This paper introduces concepts that could prove useful in the design of humanized antibodies: (1) the human framework chosen was very homologous to the original mouse antibody so as to reduce any deformation of the mouse CDRs; and (2) computer modeling was used to identify several framework amino acids in the mouse antibody that might interact with the CDRs or directly with the antigen. These amino acids were transferred to the resulting antibody.

14. • JUNGHANS RP, WALDMANN TA, LANDOLFI NF, AVADOLVIC NM, SCHNEIDER WP, QUEEN C: **Anti-tac H, a humanized antibody to the interleukin-2 receptor with new features for immunotherapy in malignant and immune disorders.** *Cancer Res* 1990, 50:1495–1502.

In this humanized antibody, 10% of the protein mass remains mouse. These segments of mouse protein are found in 6 to 9 amino acid units. The humanized antibody, produced in SV40 cells after xanthine and hygromycin selection, may actually be more active than the mouse antibody in recruiting effector function, as assayed by ADCC.

15. • NAKATANI T, NOMURA N, HORIGOME K, OHTSUKA H, NOGUCHI H: **Functional expression of human monoclonal antibody genes directed against Pseudomonal exotoxin A in mouse myeloma cells.** *Bio/Technology* 1989, 7:805–810.

Using an EBV-transformed human B-cell line, a human monoclonal antibody to exotoxin A was identified and the gene cloned. The intact gene, which includes the human IgM enhancer and promoter, was used to direct expression of this human protein in mouse myeloma cells.

16. • GILLIES SD, DORAI H, WESOLOWSKI J, MAJEAU G, YOUNG D, BOYD J, GARDNER J, JAMES K: **Expression of human antibody in transfected murine myeloma cells.** *Bio/Technology* 1989, 7:799–804.

A heterohybridoma mouse–human cell line was used as the RNA source for cDNA cloning a human antibody to tetanus toxoid. The cDNA for the heavy-chain variable region was introduced into an expression vector containing a genomic sequence for the constant region. The light-chain cDNA was expressed intact. These vectors included the immunoglobulin heavy-chain enhancer and the metallothionein promoter, and used the gpt and DHFR genes for selection.

17. • SIMON T, RAJEWSKY K: **Antibody domain mutants demonstrate autonomy of the antigen binding site.** *EMBO J* 1990, 9:1051–1056.

The main finding of this study is that the binding properties of an antibody to the hapten 4-hydroxy, 3-nitrophenyl acetyl antigen, are not changed by attaching the $V_H$–$V_L$ domain pair to C regions of different chain classes.

18. • NAKAKI T, DEANS R, LEE AMY: **Enhanced transcription of the 8,000 dalton glucose-regulated protein (GRP78) gene and association of GRP78 with immunoglobulin light chains in a non-secreting B-cell myeloma line (NS-1).** *Mol Cell Biol* 1989, 9:2233–2238.

Enhanced transcription of GRP78 or BiP is found in a myeloma cell line that produces but does not secrete light chains in the absence of heavy chain. A specific association between the light-chain molecules and BiP is discussed.

19. • TAN LK, SHOPES RJ, OI VT, MORRISON SL: **Influences of the hinge region on complement activator Clq binding, and segmental flexibility in chimeric human immunoglobulins.** *Proc Natl Acad Sci USA* 1990, 87:162–166.

This work engineers chimeric antibodies with different hinge region amino acids to study the role of the hinge region in determining the flexibility of the Fab segment. Conclusions include: (1) segmental motion correlates with the upper hinge length; (2) hinge length α segmental flexibility is not enough to alter complement binding and activation; and (3) segmental flexibility does not correlate with proficiency to activate the complement cascade.

20. • DAVIS AC, ROUX KH, PURSEY J, SHULMAR MJ: **Intermolecular disulfide bonding in IgM: effects of replacing cysteine residues in the μ heavy chain.** *EMBO J* 1989, 8:2519–2526.

Site-directed mutagenesis and recombinant antibody expression is used to address the role of disulfide bond formation in the assembly of IgM molecules. The Cys 414–Cys 414 bond is identified as playing an important role in the formation of this hexameric complex.

21. • SONGSIVILAI S, CLISSOLD PM, LACHMAN PJ: **A novel strategy for producing chimeric bispecific antibodies by gene transfection.** *Biochem Biophys Res Commun* 1989, 164:271–276.

Variable regions of a mouse antibody were cloned in vectors containing human constant genomic DNA. The subsequent bispecific antibody recognizes hepatitis surface antigen and 5-iodo-4-hydroxy-3-nitrophenyl.

22. • CAPON DJ, CHAMOU SM, MORDENTI J, MARSTERS SA, GREGORY T, MITSUYA H, BYRN RA, LUCAS C, WURM FM, GROOPMEN JE, BRODER S, SMITH DH: **Designing CD4 immunoadhesions for AIDS therapy.** *Nature* 1989, 337:525–531.

Soluble CD4 is made as a fusion protein with the IgG heavy-chain constant region. The half-life of the CD4—immunoglobulin fusion protein is increased several hundred fold over the non-fusion soluble form of CD4.

23. • TRAUNECKER A, SCHNEIDER J, KIEFER H, KARJALAINEN K: **Highly efficient neutralization of HIV with recombinant CD4-immunoglobulin molecules.** *Nature* 1989, 339:68–70.

A form of soluble CD4 is made as a fusion of the first two N-terminal domains of CD4 to the mouse γ constant region or in heavy-chain constant regions. Deletion of $C_H1$ domain of the constant region leads to more efficient secretion of the chimeric molecules.

24. • MARIUZZA RA, WINTER G: **Secretion of homodimeric $V_\alpha$ $C_K$ T cell receptor-immunoglobulin chimeric protein.** *J Biol Chem* 1989, 264:7310–7316.

This report discusses the use of protein engineering techniques to construct a chimeric T-cell receptor immunoglobulin gene encoding a secreted soluble form of the variable domain of a human T-cell receptor α chain. These experiments fuse the T-cell receptor specific for diphtheria toxoid to a human κ light-chain constant region.

## Annotated patents

- • Of interest
- •• Of outstanding interest

P1 • MASSACHUSETTS INSTITUTE OF TECHNOLOGY AND WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH: **New constitutive and tissue-specific protein factors which bind to transcriptional regulating elements of IGG genes.** 12/2/88 88US-155207. 24/8/89 WO89 07614 A.

Four transacting proteins NF-A1, NF-AZ, E factors and NF-κB were identified and isolated by an improved assay for protein–DNA binding. These proteins, which are constitutive and tissue-specific factors should enable cloning of a gene that can be used to regulate immunoglobulin transcription.

P2 • BRISTOL-MEYERS COMPANY: **Production of chimeric antibodies by homologous recombination using a target vector comprising a replacement and a homologous target sequence.** 27/10/87 87US-113800. 10/5/89 EP-315062 A.

Chimeric antibodies are produced by transfecting human IgG1 constant regions into antibody-producing lines. Through homologous recombination, the endogenous murine constant regions are replaced with the human sequence. The frequency of integration events that resulted in human IgG production was 0.75%.

P3 • ELI LILLY & CO: **Novel recombinant and chimeric antibodies directed against a human adenocarcinoma antigen.** 21/4/88. 25/10/88 US 184522.

The expression vectors described here use the BK enhancer with the adenovirus late promoter to direct transcription of the chimeric antibody cDNA. An intron is included 3′ of the cDNA. Non-myeloma cells are used for expression of the antibodies.

P4 • CELL TECH LIMITED: **Recombinant antibodies and methods for their production.** 9/3/89 WO 89/01975.

This invention describes mammalian cell expression of recombinant, including chimeric or humanized, IgM-type antibodies in non-lymphoid-type cells. The IgM complex is formed even in the absence of the J chain which is endogenous in lymphoid cells but absent in other cell types. A preferred use of this system is the expression of an IgM antibody in glioma cells; this expression of antibodies in glioma cells could enable recombinant cellular transplantation into the brain.

# Other topics in mammalian cell expression

As the foregoing review focuses on the expression of recombinant antibodies, other interesting developments in mammalian gene expression are covered by the following annotated papers and patents.

## Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

### Amplification and selection

ANDERSON KP, LIE YS, LOW ML, WILLIAMS SR, FENNIE EH, • NGUYEN TP, WURM FM: **Presence and transcription of intracisternal A-particle-related sequences in CHO cells.** *J Virol* 1990, 64:2021–2031.

Chinese hamster ovary cell (CHO) sequences related to intracisternal A particles (IAP) genes of mice and Syrian hamster. Clones homologous to Syrian hamster IAP have been isolated and used to evaluate the abundance and expression pattern of the retrovirus-like sequences in CHO. These IAP-related sequences appear as a moderately repetitive family with about 300 copies per genome. Two families of repetitive sequences have been identified.

GERMANN UA, CHIN KV, PASTAN I, GOTTESMAN MM: Retrovi- • **ral transfer of a chimeric multidrug resistance—adenosine deaminase gene.** *FASEB J* 1989, 4:1501–1507.

A fusion protein containing sequences from two selectable marker cDNAs, multidrug resistance (MDR) and adenosine deaminase (ADA), establishes both phenotypes simultaneously. *In vitro*, and *in vivo* studies using retroviral transfer followed by colchine selection produce the MDR–ADA fusion. This protein is stably expressed and exhibits bifunctional biological activity.

GERMANN UA, GOTTESMAN MM, PASTAN I: **Expression of a mul-** • **tidrug resistance adenosine deaminase.** *J Biol Chem* 1989, 264:7418–7424.

A novel fusion gene has been created in which the expression of a dominant selectable marker is directly linked to the expression of a second marker cDNA. A membrane-associated human P-glycoprotein–ADA fusion protein is synthesized which retains function of the multidrug transporter and exhibits ADA activity.

KOHN DB, MITSUYA H, BALLOW M, SELEGUE JE, BARAN KIEWEIZ • J, COHEN A, GELFORD E, ANDERSON WF, BLAESE RM: **Establishment and characterization of adenosine deaminase deficient human T cell lines.** *J Immunol* 1989, 142:3971–3977.

This report describes the establishment and characterization of T-cell lines from a patient with ADA-deficient severe combined immunodeficiency (SCID) by the use of the human-transforming retrovirus HTLV-1. These cells may be valuable for examining the role of ADA in normal metabolism and for establishing cell lines that can be complemented by expression of the ADA gene.

MIELE M, BONATTI S, MENICHINI P, OTTAGGIO L, ABBONDANDOLO • A: **The presence of amplified regions affects the stability of chromosomes in drug-resistant Chinese hamster cells.** *Mutation Res* 1989, 219:171–178.

Chinese hamster cells were subjected to amplification regiments for either carbamyl phosphate synthetase-aspartate transcarbomylase dihydrooratase (CAD) or DHFR. In both cases, amplified DNA was observed in micronuclei, and displaced chromosomes were observed at various times during mitosis. Chromosomes bearing an amplified region tended to be excluded from cells, and rearrangements were more frequent than in normal chromosomes.

PALLAVICINI MG, DE TERESA O, ROSETTE C, GRAY JW, WURM • FM: **Effects of methotrexate on transfected DNA stability in mammalian cells.** *Mol Cell Biol* 1990, 10:401–404.

Fluorescence *in situ* hybridization is used to characterize the pattern of integration of DNA followed DHFR amplification. Most of the cells containing highly amplified amounts of transfected DNA were stable and lost these transfected sequences when MTX was removed.

SAITO I, GROVES R, GUILOTTO E, ROLFE M, STARK GR: Evolution • **and stability of chromosomal DNA coamplified with the CAD gene.** *Mol Cell Biol* 1989, 9:2445–2452.

During amplification of the CAD gene in Syrian hamster cells both primary and secondary amplification events are seen to be unstable in the absence of selection.

SRIMATKANDADA S, SCHIVETZER BI, MOROSON BA, DUBE S, • BERTINO JR: **Amplification of a polymorphic dihydrofolate reduction gene expressing an enzyme with decreased binding to methotrexate in the human colon carcinoma cell line, HCT-8R4, resistant to this drug.** *J Biol Chem* 1989, 264:3524–3528.

A MTX-resistant human colon cell line was found to have both amplified and mutated DHFR.

STARK GR, DEBATISSE M, GUILOTTO E, WAHL GM: Recent • **progress in understanding mechanisms of mammalian DNA amplification.** *Cell* 1989, 57:901–908.

A good discussion of the current ideas behind the mechanisms of DNA amplification.

STOCKINGER H, KUBBRIS M: **Cell cycle-dependent DHFR and** • **tPA production in cotransfected MTX-amplified CHO cells revealed by dual-loser flow cytometry.** *Exp Cell Res* 1990, 188:267–271.

Shows that CHO cells amplified for both DHFR and tissue plasminogen activator (tPA) exhibit different cell-cycle-correlated accumulation and secretion of tPA and production of DHFR. Therefore, the highest fluorescent MTX-positive cells do not necessarily display the highest tPA secretion rate.

THELANDER M, THELANDER L: **Molecular cloning and expres-** • **sion of the functional gene encoding the M2 subunit of mouse ribonucleotide reductase: a new dominant marker gene.** *EMBO J* 1989, 8:2475–2479.

Transfection of mouse cells with the M2 subunit of the ribonucleotide reductase gene results in stable transformants with a 10-fold reduction in sensitivity to hydroxyurea.

TRASK B, HAMLIN JL: **Early dihydrofolate reductase gene am-** • **plification events in CHO cells usually occur on the same chromosome arm as the original locus.** *Genes Dev* 1989, 3:1913–1925.

Fluorescence *in situ* hybridization is used to examine the early stages of DHFR DNA amplification. Amplified DHFR sequences were found to occur most often on the same chromosome arm as the parental DHFR gene, but at a considerable distance from it. These results support either sister chromatid exchange between widely separated sites or a form of conservative intrachromosomal duplication as the mechanism for amplification.

### Techniques

BELT PBGM, GROENEVELD H, TENBEL WJ, VAN DE PATTE, • BACKENDORF C: **Construction and properties of an Epstein-Barr virus derived cDNA expression vector for human cells.** *Gene* 1989, 84:407–417.

The presence of EBV sequences, ori P and EBNA-1 resulted in five-to-tenfold increase in transfection efficiency when compared with non-epi-

somal vectors. Transfections performed with a mixture of EBV vectors transfectants are seen to contain more than one plasmid type.

• BERU N, MCDONALD J, GOLDWASSER E: **Activation of the erythropoietin gene due to the proximity of an expressed gene.** *DNA* 1989, 8:253–259.

In the cell line IW32 an erythropoietin gene is transcribed from an upstream unclassified promoter from a read-through transcript. This upstream region shows the characteristic DNase sensitivity seen with actively transcribed chromatin.

• CLEMENT JM, JEHANNO M, HOFNUNG M: **β-galactosidase over expression in SV40 transformed Chinese hamster fibroblasts exposed to mutagens as a result of amplification of transfected bacterial lac Z DNA sequences.** *Mutation Res* 1989, 218:179–188.

Cell lines were designed in which genotoxic agents induce the synthesis of β- galactosidase through increased copy number of DNA segments containing the SV40 origin and the structural gene for β-galactosidase.

• GOULD-FOGERITE S, MAZURKIEWICZ JE, RASHA K, VOEDKERDING K, LEHMAN JM, MANNINO RS: **Chimerasome-mediated gene transfer *in vitro* and *in vivo*.** *Gene* 1989, 84:429–438.

DNA encapsulated in chimeric proteoliposome gene-transfer vesicles, or chimerasomes, can greatly increase transfection efficiency over that seen with calcium phosphate precipitation.

• IKEHATA H, KAMURA H, KATO T: **Shuttle vector system for the analysis of mutational events in mammalian chromosomal DNA.** *Mutation Res* 1989, 210:237–247.

This study introduces the hprt gene in a retroviral vector (pZipneoSVXI) into the chromosome of a hprt-deficient cell line. The integrated DNA is rescued for mutational analysis of hprt following fusion with COS cells.

• ISHURA M, OHASHI H, USHIDA TB, OKADA V: **Phage particle-mediated gene transfer of recombinant cosmids to cultured mammalian cells.** *Gene* 1989, 82:281–289.

Purified packaged cosmids were introduced into cells by the phage transfer method. The packaged cosmids were transferred to Ltk cells as efficiently as recombinant phages.

• JALANKO A, KALLIO A, SALMINER M, ULMANEN I: **Efficient synthesis of influenza virus hemagglutinin in mammalian cells with an extra chromosomal Epstein-Barr vector.** *Gene* 1989, 78:287–296.

An EBV based episomal vector using the human cytomegalovirus enhancer and the SV40 promoter to direct expression of hemagglutinin was tested in human and monkey cells. A fluorescent activated cell sorter (FACS) was used to select clones expressing the recombinant protein on the cell surface. High levels of expression were achieved.

• KEATING A, HORSPALL W, HAWLEY RG, TONEGUZZO F: **Effect of different promoters on expression of genes introduced into hematopoietic and marrow stromal cells by electroporation.** *Exp Hematol* 1990, 18:99 102.

The murine cytomegalovirus promoter is shown to be highly active in hematopoietic progenitor cells. These stably transfected stromal cells may be an attractive vehicle for human gene therapy.

• KO MSH, TAKAHASHI N, SUGUYAMI N, TAKANO T: **An auto-inducible vector conferring glucocorticoid inducibility upon stable transformant cells.** *Gene* 1989, 84:383–389.

Mouse Ltk-cells were transfected with the glucocorticoid receptor under control of the glucocorticoid-inducible enhancer/promoter of the mouse mammary tumor virus. The receptor was induced by dexamethosome. This led to a greater than 100-fold increase in expression of a reporter gene under control of the mouse mammary tumor virus.

• KO MSH, TAKANO T: **A highly inducible system of gene expression by positive feedback production of glucocorticoid receptors.** *DNA* 1989, 8:127–133.

This expression system increases the concentration of the glucocorticoid receptor via a positive feedback response using the hormone-inducible signals to control transcription of the receptor gene. Coexpression of this plasmid can augment the inducible expression level of ordinary glucocorticoid-inducible genes without affecting basal expression levels.

• MARSCHALL M, MOTZ M, LESER U, SCHWARNMANN F, OKER B, WOLF H: **Hepatitis B virus surface antigen as a reporter of promoter activity.** *Gene* 1989, 81:109–117.

The secreted protein hepatitis-B surface antigen is evaluated as a transient assay marker. It has the advantage of being detectable by readily available diagnostic assay kits. Because the protein is secreted, the assay leaves the cell intact for further analysis.

• PALUDON K, DUCH M, JORGENSON P, KJELDGAARD NO, PEDERSON FS: **Graduated resistance to G418 leads to differential selection of cultured mammalian cells expressing the new gene.** *Gene* 1989, 85:421–426.

Comparison of vector-positive populations, before and after selection, showed different expression levels and copy number distribution for populations infected with a vector with low long terminal repeat activity.

• PEDEN KWC, CHARLES C, SANDERS L, TENNEKOM GI: **Isolation of rat Schwann cell lines: use of SV40 T antigen gene regulated by synthetic metallothionein promoters.** *Exp Cell Res* 1989, 185:60–72.

Promoter elements from the mouse metallothioncin promoter were used to express SV40 T antigen in rat Schwann cells. Clones were obtained that were immortalized, yet retained the characteristics of untransfected Schwann cells.

• ROGERS P, FISHER R, GUYDES J: **Neutral selection of transfected mammalian cells using tissue plasminogen activator gene expression.** *Gene* 1989, 81:151–158.

Expression of tPA is used as a selection system. The detection assay for expression, caseinolysis, leaves the cells intact and single cell transfectants are easily identified.

• YOUFUJI T, MIKAWA H: **Co-transfer of restriction endonucleases and plasmid DNA into mammalian cells by electroporation: effects on stable transformation.** *Mutation Res* 1990, 243:121–126.

The cloned herpes simplex virus thymidine kinase gene was introduced into Ltk-mouse cells with restriction endonucleases. A stimulation in transformation efficiency was observed for various forms of exogenous DNA. The presence of DNA double-stranded breaks, induced by the cotransfected enzymes, stimulates the integration of exogenous DNA into host cell.

## Glycosylation studies

• HAMAGUCHI M, TAKAHASI I, TAKEHARS T, TAKAMATSU J, SAITO H: **Comparison of recombinant tissue-type plasminogen activator (rtPA) expressed in mouse C127 cells and human vascular plasminogen activator (HV-PA).** *Biochem Biophys Acta* 1989, 1009:143–150.

The composition and activity of recombinant tPA produced in mouse C127 cells is compared with tPA of human origin.

• KONIG R, ASHWELL G, HANMER J: **Over expression and biosynthesis of CD4 in Chinese hamster ovary cells: coamplification using the multiple drug resistance gene.** *Proc Natl Acad Sci USA* 1989, 86:9188–9192.

The MDRI selectable marker is used to introduce the CD4 gene into both wild-type and glycosylation-deficient mutant CHO cells. The sensitivity of CD4 to glycosidases suggested the presence of bi-antennary unsialylated complex-type oligosaccharides.

• PAREKH RB, DWEK RA, RUDD PM, THOMAS JR, RADEMACHER TW, WARREN T, WUN RC, HERBERT B, REITZ B, PALMER M, RAMABHADRAN T, TIEMEIER DC: **N-glycosylation and *in vitro* enzymatic activity of human recombinant tissue plasminogen activator expressed in Chinese hamster ovary cells and a murine cell line.** *Biochemistry* 1989, 28:7670–7679.

tPA is expressed in either CHO cells using DHFR selection or in mouse C127 cells using a bovine papilloma vector and the neomycin resistance gene. The results support the idea that N-glycosylation is cell-type specific.

• TAKEUCHI M, INOUE N, STRICKLAND GTW, KOBATA M, WADE M, SHIMIZU R, HOSHI S, KOZUTSUMI H, TAKASAKI S, KOBATA A: **Relationship between sugar chain structure and biological**

activity of recombinant human erythropoietin produced in Chinese hamster ovary cells. *Proc Natl Acad Sci USA* 1989, 86:7819–7822.

Examination of different preparations of recombinant human EPO produced in CHO cells showed a good correlation between *in vivo* activity of EPO and the rates of tetra-antennary to bi-antennary oligosaccharides.

• YANAGI H, YOSHIMA T, OGAWA I, OKAMOTO M: **Recombinant human erythropoietin produced by namalwa cells.** *DNA* 1989, 8:419–427.

The cDNA coding for the human erythropoietin gene was expressed under control of the SV40 promoter in the human B-lymphoblastoid cells. Analysis of carbohydrates showed the oligosaccharide structure of recombinant erythropoietin to be similar to that of urinary erythropoietin.

• ZANN EE, KOUVATSI A, HADZOPOULOH G, CLADARAS M, KREIGER M, ZANNIS V: **Expression of Apo E gene in Chinese hamster cells with a reversible defect in O-glycosylation. Glycosylation in not required for apoE secretion.** *J Biol Chem* 1989, 264:9137–9140.

The effects of O-glycosylation in the synthesis and secretion of Apo E were studied in a CHO cell line that cannot add N-acetylgalactosamine. Data suggest the addition of galactose to the nascent oligosaccharide chains is required for the addition of sialic acid.

## General expression

• CURTIS BM, GALLIS B, OVERELL RW, MCMAHON CJ, DE ROOS P, IRELAND R, EISENMAN J, DOWER SK, SIMS JE: **T-cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to interleukin 1.** *Proc Natl Acad Sci USA* 1989, 86:3045–3049.

A cDNA encoding a receptor that binds interleukin 1α and β in murine T cells is stably established in wild-type CHO cells using the neomycin-resistance marker. Results presented show that the interleukin cDNA encodes the entire functional receptor and that the cytoplasmic domain is required for signal transduction but not ligand binding.

• FRASER CM, ARAKAWA S, MCCOMBIE WR, VENTER JC: **Cloning sequence analysis and permanent expression of human $\alpha_2$-adrenergic receptor in Chinese hamster ovary cells.** *J Biol Chem* 1989, 264:11754–11761.

The gene encoding a human α2-adrenergic receptor was isolated from a human genomic type DNA library and expressed in wild-type CHO cells using the neomycin selectable marker. Data present suggest that the human α2-adrenergic receptor in CHO cells may simultaneously couple to more than one effector.

• GEARING DP, KING JA, GOUGH NM, NICOLA NA: **Expression cloning of a receptor for human granulocyte-macrophage colony stimulating factor.** *EMBO J* 1989, 8:3667–3676.

The receptor for human GM-CSF is isolated by expression screening a library from human placental mRNA expressed in COS cells.

• JOHNSON EF, WALKER DL, GRIFFIN KJ: **Cloning and expression of three rabbit kidney cDNAs encoding lauric acid hydroxylases.** *Biochemistry* 1990, 29:873–879.

cDNAs encoding three cytochrome P-450 enzymes were cloned from a rabbit kidney cDNA library and transiently expressed in COS cells. Results indicate that the substrate selectivity of the kidney enzyme is distinct from the lung enzyme.

• KEENE JL, MATZUK MM, OTANI T, FANSER BCJM, GALWAY AB, HSUEH AJW, BIOME I: **Expression of biologically active follitropin in Chinese hamster ovary cells.** *J Biol Chem* 1989, 264:4769–4775.

The follitropin β subunit is secreted very slowly from CHO cells transfected with β subunit DNA alone. Cotransfection with the α subunit DNA results in rapid secretion of dimer.

• LEIBROCK J, LOTTSPEICH F, HAHN A, HOFER M, HENGERER B, MASIAKOWSKI P, THOENEN H, BARDE YA: **Molecular cloning and expression of brain-derived neutrophotic factor.** *Nature* 1989, 341:149–152.

Brain-derived neurotrophic factor was cloned by PCR amplification and the full length cDNA transiently expressed in Cos cells.

• LOWE DG, NUNES W, BOMBARA M, MCCABE S, RANGES GE, HENZEL W, TOMIDA M, YAMAGUCHI YY, HOZUMI M, GOEDDEL DV: **Genomic cloning and heterologous expression of human differentiation-stimulating factor.** *DNA* 1989, 8:351–359.

Differentiation factor from mouse ascite cells was purified and the gene cloned and expressed in COS cells. In terms of both sequence and activity, the differentiation factor is shown to be identical to leukemia inhibitory factor.

• MADISEN L, FARRAND AL, LEOUBIN MN, MARZOWSKI J, KNOX LB, WEBB NR, LIM J, PUNCHIO AF: **Expression and characterization of recombinant TGFB2 proteins produced in mammalian cells.** *DNA* 1989, 8:205–212.

CHO cells transfected with a fusion of transforming growth factor (TGF)B1 amino-terminus and TGFB2 a carboxyterminus secretes both precursor and mature forms of the protein. Processing signals contained within the TGFB1 amino portion function to produce mature TGFB2.

• NAKAMURA T, NISHIZAWA T, HAGIYA M, SEKI T, SHIMONISHI M, SUGIMURA A, TASHIRO K, SHIMIZA S: **Molecular cloning and expression of human hepatocyte growth factor.** *Nature* 1989, 342:440–443.

The report details the amino acid sequence for hepatocyte growth factor determined by cDNA cloning and the expression of biologically active human hepatocyte growth factor from transiently transfected COS cells.

• STEIN C, HILLE A, SEIDEL J, RYNBOUT S, WAHRED A, SCHMIDT B, GEUZE H, VAN FIGINE K: **Cloning and expression of human α steroid sulfatase.** *J Biol Chem* 1989, 264:13865–13872.

The cDNA for human steroid sulfatase was expressed in COS and baby hamster kidney cells. The transport of this protein was followed through cellular organelles in the hamster cells.

# Annotated patents

- • Of interest
- •• Of outstanding interest

## Amplification and selection

• BIOGEN INC.: **Improved electroporation method for stable integration of foreign DNA at high copy number.** 29/11/89 88US-185212 EP-343783A.

The invention describes the use of electroporation to introduce plasmid DNA into eukaryotic cells. The plasmid preferably contains a dominant selectable marker, the gene of interest and an amplifiable marker.

• CODON CORPORATION: **Gene amplification in eukaryotic host cells using expression vector comprising wild-type amplifiable gene and structural gene.** 30/11/87 87US-126436. 7/6/89 B EP-319206 A.

Describes amplification using non-mutant host cells. Two schemes are described: use of a selectable marker to isolate clones followed by amplification for a second gene, and the direct use of amplification in wild-type host cells.

• CODON CORPORATION: **Supertransformants for high expression rates in eukaryotic cells.** 10/1/89. WO 89/10959.

This invention describes a protocol for transfecting a eukaryotic cell with one selectable marker and the gene of interest followed by retransfection with the gene of interest and a second selectable marker. It enables the copy number of the gene of interest to be increased without requiring amplification.

CELLTECH LIMITED: **Recombinant DNA methods, vectors and host cells.** 25/10/89 1804. GB89809129.

Transformation of a lymphoid cell line to glutamine independence is described using the glutamine synthease gene. This method allows the culturing of resultant clones in the absence of exogenous glutamine. Increasing amounts of metheonine sulphoxemine can be used to select for cells that have amplified the glutamine synthease gene.

## Techniques

COMMONWEALTH SCIENTIFIC ORGANIZATION: **Gene expression system especially for rotavirus VP7 protein using a foreign signal peptide and optionally a transmembrane anchor sequence.** 5/2/88 88AU-006612. 10/8/89 WO8907140 A.

Describes the expression and export of proteins and membrane anchoring of proteins; these are normally intracellular. This patent specifically addresses the production of a membrane-bound form of retrovirus VP7 protein for use as a vaccine.

ELI LILLY & CO.: **Vectors and compounds for direct expression of activated human protein C.** 7/6/89 US 129027.

The method described involves transformation and culture of mammalian cells in order to produce protein C. In this method the activation peptide is replaced with a cleavage sequence for a cell-associated protease.

VANDERBILT UNIVERSITY: **Functional mutated EIA gene of human adenovirus used to express EIA products that stimulate promoters controlling the EIA.** 4/1/88 88US-140625. 13/7/89 WO8906282 A.

Mutated EIA genes of human adenovirus subgroup B1 that do not repress enhancers were isolated. The auto-repression domain is between nucleotides 956 and 1024. These mutants should facilitate promoter activity without enhancer repression.

MOLECULAR THERAPEUTICS INC.: **Transfectant cell lines which express the major human rhinovirus receptor.** 14/6/89 8/12/87 US 130378.

HeLa cell DNA is used as the source of the expression library. Primary transfectants were screened by FACS using a fluorescence-based virus-binding assay. Secondary transfectants were made in order to select for high-expression cell lines.